

# Bulletin of the Agricultural Chemical Society of Japan.

## TRANSACTIONS

### Biochemistry of Filamentous Fungi. VI.

Mycelial Constituents of *Oospora sulphurea-ochracea*. Part III.

Trimethylsulochrin and its Fission Products.

By Hidejiro NISHIKAWA.

(Tottori Agricultural College.)

Received May 11, 1940.

Of the crystalline constituents of the mycelium of *Oospora sulphurea-ochracea* described in the previous communication,<sup>(1)</sup> sulochrin (substance B) was proved by further experiments to be methyl ester of 2:6:4'-trihydroxy-4-methyl-6'-methoxy-benzophenone-2'-carboxylic acid, the structural discussion having been advanced in detail in a separate paper.<sup>(2)</sup>

Of the three hydroxyl groups which are present in the sulochrin molecule two could be readily methylated by means of diazomethane; one of the two hydroxyl groups in the *p*-orsellinic moiety situated at ortho-position to the central carbonyl resisted this means of methylation.

The fully methylated derivative has now been prepared by repeated application of dimethylsulphate on sulochrin. Trimethylsulochrin thus obtained can be split neatly into two halves by means of conc. sulphuric acid followed by the addition of water, just as in the case of sulochrin and dimethylsulochrin, resulting fragments being, as is anticipated, dimethyl-*p*-orsellinic acid and methyl dimethyl- $\alpha$ -resorcylate and thus giving an additional evidence of the structure of sulochrin. A more drastic measure of hydrolysis, however, is required in this case. While in the case of sulochrin and dimethylsulochrin extreme decomposition had to be controlled by ice-cooling the mixture, for the complete hydrolysis of trimethylsulochrin it was necessary to warm the sulphuric acid solution for some time on a water-bath.

Action of methyl alcoholic potash on trimethylsulochrin yields monomethyl-tetramethoxy-benzophenonecarboxylic acid which is isomeric with dimethylsulochrin, one methoxyl of ester-form having been lost.

## EXPERIMENTAL.

Trimethylsulochrin (4-methyl-2 : 6 : 4' : 6'-tetramethoxy-  
2'-carbomethoxybenzophenone).

Five grams of sulochrin was dissolved in 12.5 cc of 10% NaOH and shaken vigorously on a machine, 15 cc of dimethyl sulphate and 77.5 cc of 10% NaOH being alternately dropped in during three hours. Amorphous solids separated and were collected, yield nearly quantitative. One gram of the crude material, which contained incompletely methylated impurities, was dissolved in acetone, 2 cc of dimethyl sulphate was added and, while shaking, 12 cc of 10% NaOH was added drop by drop. To the resulting clear solution much water was added till the precipitation of crystals occurred (yield nearly quantitative) which melted at 152°. On recrystallization from benzene the substance crystallized in platelets and melted at 157°, turning pink. The melting-point was quite similar to that of dimethylsulochrin and to make sure of their dissimilarity a mixed melting-point was taken which showed marked depression. (Found: C, 64.40; H, 5.94%.  $C_{20}H_{22}O_7$  requires C, 64.17; H, 5.88%. Methoxyl. Found: 40.93%. 5(CH<sub>3</sub>O) in  $C_{20}H_{22}O_7$  requires 41.44%).

Trimethylsulochrin dissolves readily in acetone, chloroform, methyl alcohol, ethyl acetate, moderately in ethyl alcohol and benzene, slightly in ether, but not in light petroleum and water. It does not give a FeCl<sub>3</sub> reaction.

## Fission of trimethylsulochrin by means of conc. sulphuric acid.

To 2.4 g of trimethylsulochrin was added 24 cc of conc. sulphuric acid and the mixture was warmed on a water-bath until the initial dark brown colour of the solution changed into a deep reddish purple tint. The whole matter was then poured into a large amount of water, the resulting emulsion being thoroughly extracted with ether. The ether layer was shaken twice with 5% bicarbonate solution, this in turn was acidified and again extracted with ether. The ether solution was dried and distilled, 0.7 g substance being left behind. For purification it was dissolved in methyl alcohol and precipitated by the addition of water. It melted at about 165° and gave a green FeCl<sub>3</sub> reaction. The crystals were again dissolved in a small quantity of warm methyl alcohol and cooled with ice, when beautiful square platelets devoid of FeCl<sub>3</sub> reaction separated out. The melting point now rose to 182° and mixed melting point with a synthetic specimen of dimethyl-p-orsellinic acid showed no depression.

The fraction insoluble in bicarbonate, after distilling off the solvent, was left behind as a yellowish oil (0.7 g), in which was seeded a tiny fragment of methyl dimethyl- $\alpha$ -resorcylate. A magma of large needle crystals separated which melted at 44° alone or mixed with an authentic specimen of methyl- $\alpha$ -resorcylate.

## 4-Methyl-2:6:4':6'-tetramethoxy-2'-carboxybenzophenone.

Trimethylsulochrin (1 g) was boiled with methyl alcohol (30 cc) and KOH (1 g) under reflux for five hours. On cooling it was diluted with water and acidified with HCl, when the liquid became turbid and gradually separated prism crystals (0.75 g). It melts at 194° and gives neither  $\text{FeCl}_3$  nor  $\text{CaOCl}_2$  reaction. (Found: C, 63.04; H, 5.61%.  $\text{C}_{19}\text{H}_{20}\text{O}_7$  requires C, 63.33; H, 5.56%. Methoxyl Found: 34.11%. 4( $\text{CH}_3\text{O}$ ) in  $\text{C}_{19}\text{H}_{20}\text{O}_7$  requires 34.44%). It readily dissolves in acetone, methyl alcohol, chloroform, moderately in ethyl alcohol, less in ethyl acetate and benzene, sparingly or not in light petroleum, ether and water.

## REFERENCES.

- (1) Nishikawa: Bull. Agr. Chem. Soc. Jap., **13**, 1 (1937).
- (2) Nishikawa: Acta Phytochim., **11**, 167 (1939).

**ABSTRACTS**

from

**TRANSACTIONS published in JAPANESE**

(Pages refer to the Japanese originals of this volume unless otherwise noted.)

**On the Stimulant for Cane Sugar Formation  
in Plants. (VI)**

(pp. 501~503)

By Tetutara TADOKORO and Masao NISIDA.

(Hokkaido Imperial University; Received May 14, 1940.)

**Separation and Identification of Fatty Acids.**

(pp. 504~512)

By Y. INOUE and H. YUKAWA.

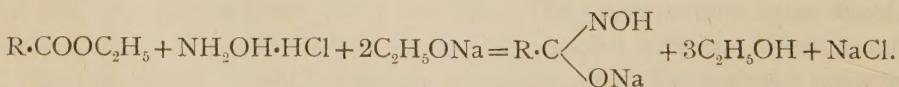
(Agr. Chem. Laboratory, Kyoto Imp. Univ.; Received May 9, 1940.)

**Part I. Hydroxamic Acids derived from Saturated Fatty Acids.**

For making a research of the chemical structures of fats and oils or their fatty acids, we must establish the methods for the pure separation of each acid. At least it is requisite to isolate each fatty acid in the crystal state.

Hydroxamic acids which can be obtained from fatty acids by reaction with hydroxylamine are crystal substances with relatively higher melting points. Therefore, we have made the fundamental experiments utilizing these properties.

Although there were several methods used to obtain hydroxamic acid, we studied the reaction of esters or glycerides with hydroxylamine in the presence of sodium ethylate in order to prepare it directly from fats and oils. The reaction was as follows:



This reaction proceeded quantitatively at room temperature without moisture.

Hydroxamic acids derived from saturated fatty acids which were obtained in the form of white crystals gave an intensive reddish-violet colour in alcohol with ferric chloride and gave green, voluminous and amorphous precipitation of copper salt in an excess alcoholic solution of copper acetate. And also we could recover the original fatty acid by refluxing the hydroxamic acid with diluted alcoholic

solution of sulphuric acid. The solubility was different in several organic solvents according to the carbon numbers of hydroxamic acids.

Table. Melting points and solubilities of hydroxamic acids.

Fatty acid	Fatty acid M. P. °C	M. P. °C	Hydroxamic acid				
			Solubility				
			Ethanol	Aceton	Ether	Water	Petr.-ether
C <sub>22</sub>	84	112.5	+	+	-	-	-
C <sub>20</sub>	77	109.5~110	+	+	-	-	-
C <sub>18</sub>	71.5~72	106.5~107	##	##	-	-	-
C <sub>16</sub>	63.5~64	102.5	##	##	-	-	-
C <sub>14</sub>	57.5~58	98~98.5	##	##	+	-	-
C <sub>12</sub>	47.5~48	94	##	##	+	-	-
C <sub>10</sub>	31.5	88~88.5	##	##	##	+	-
C <sub>8</sub>	16	78.5~79	##	##	##	+	-
C <sub>6</sub>	- 1.5	63.5~64	##	##	##	##	-
C <sub>4</sub>	- 4.7	syrup	##	##	+	##	-
C <sub>3</sub>	-19.7	92.5~93	##	##	+	##	-
C <sub>2</sub>	16.7	88	##	##	+	##	-

The increasing number of (+) means greater solubility and the (-) insoluble.

## Part II. Hydroxamic Acids derived from Unsaturated Fatty Acids.

In the previous work we derived hydroxamic acids from saturated fatty acids which contained an even number of carbon atoms, C<sub>2</sub>~C<sub>22</sub>, and decided their melting points. In the present work we obtained olein-, linol-, and linolenhydroxamic acids in the form of white crystals by the reaction of hydroxylamine to their ethyl esters in the presence of sodium ethylate, as before.

These were soluble in organic solvents, possible to recrystallize from petroleum ether, gave an intensive reddish-violet colour in alcohol with ferric chloride and gave green, voluminous, amorphous precipitation of copper salt with an excess alcoholic solution of copper acetate. Also the original fatty acid could be recovered by means of refluxing the hydroxamic acid with diluted alcoholic solution of sulphuric acid. Their melting points were 61°, 41~42° and 37~38°. The inclination of solubility in petroleum ether was olein-<linol-<linolenhydroxamic acid.

**On the Teratologic Forms of *Aspergillus Awamori* var. *fumeus*.**

(pp. 513~518)

By Matazo ABE.

(Scientific Laboratory of Ch. Takeda & Co. Ltd., Osaka; Received May 15, 1940.)

During the morphological studies of *Asp. Awamori* var. *fumeus* Nak., Sim. et Wat. the following facts have been observed:

- 1). A "white wooliness" of the fungus originates from its mycelium and sterile hyphae.
- 2). Abnormal conidiophores are produced from this mycelium.
- 3). Sterile hyphae are formed by the proliferation of some of the secondary sterigmata.
- 4). Some normal conidiophores bear a sort of abnormal forms growing parasitically in the stalks and the vesicles.
- 5). The greater part of these parasitic forms grow out into abnormal conidiophores which, in turn, produce conidia both within and without the "host."

**Studies on the Nutritive Value of Weeds.**

(pp. 519~527)

By Gôiti FUKAI.

(Agricultural Chemical Laboratory, Tokyo Imperial University;  
Received May 21, 1940.)

**I. Carotine and Vitamin C Contents and Their Fluctuations.**

**II. Vitamin B<sub>1</sub> and B<sub>2</sub> Contents.**

**Nutritional Conditions of the Wild Grazing Horse.**

(pp. 528~530)

By Gôiti FUKAI.

(Agricultural Chemical Laboratory, Tokyo Imperial University;  
Received May 21, 1940.)

## Biochemical Studies on "Bakanae" Fungus of Rice. Part VI.

Effect of gibberellin on the activity of amylase in germinated cereal grains.

(pp. 531~538)

By Takesi HAYASI.

Imperial Agricultural Station; Received May 8, 1940.)

Gibberellin, the active principle which makes the rice seedlings grow abnormally tall, has stimulative action on the germination of barley (hulled and naked), wheat and rice grains and on the activity of amylase in germinated barley (hulled and naked) and wheat grains.

## Studies on the Fibres of the Skinfat-layers of the Whale.

(pp. 539~540)

By Tosio NAKAHAMA and Masao HASEGAWA.

(Kanebo Yamashina Institute; Received May 21, 1940.)

## Über die Verwitterung der Eruptivgesteine. VII.

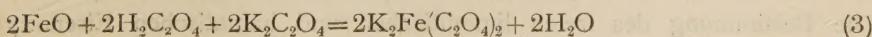
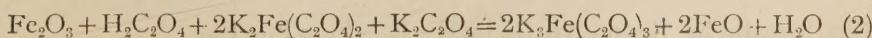
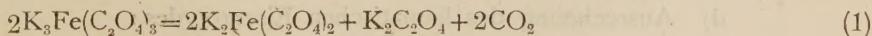
Eine neue Methode zur Bestimmung des freien Eisenoxydes.

(SS. 541~551)

Von Mituru HARADA.

(Landwirtschaftliche Hochschule Tottori; Eingegangen am 20. 5. 1940.)

Wie der Verf. in der Mitteilung II und IV berichtet hat, löst sich limonitisches und hämatisches Eisenoxyd in einer schwach oxalsauren Kaliumoxalatlösung unter der Wirkung des Lichts nach einigen Stunden plötzlich auf. Der Chemismus dieser Reaktion liegt darin, dass sich zunächst eine sehr kleine Menge des Eisenoxydes löst und  $K_3Fe(C_2O_4)_3$  entsteht, das sich dann photochemisch nach der Gleichung (1) in  $K_2Fe(C_2O_4)_2$  umwandelt. Alsdann erfolgt die Auflösung des Eisenoxydes mit großer Reaktionsgeschwindigkeit unter der katalytischen Wirkung des  $K_2Fe(C_2O_4)_2$  nach dem Schema (2) und (3). Diese Reaktionen gehen unter der Einwirkung der violetten und der ultravioletten Strahlen vor sich; beim Erwärmern findet die Reaktion (2) und (3) auch im Dunkeln statt.



Auf diesen Reaktionen hat der Verf. eine neue Methode zur Bestimmung des freien Eisenoxydes und der Trennung des hämatischen und des limonitischen Eisenoxydes aufgebaut.

#### REAGENZIEN.

- (1) Oxalsäure-Kaliumoxalatlösung I : 0,025 g-Mol Oxalsäure und 0,1 g-Mol Kaliumoxalat werden zu 1000 ccm gelöst.
- (2) Oxalsäure-Kaliumoxalatlösung II : 0,005 g-Mol Oxalsäure und 0,015 g-Mol Oxalsäure und 0,015 g-Mol Kaliumoxalat werden zu 1000 ccm gelöst.
- (3) Ammonium-Ferrosulphatlösung : 17,5 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 9 g  $(\text{NH}_4)_2\text{SO}_4$  und 10 cc n/10  $\text{H}_2\text{SO}_4$  werden zu 250 ccm gelöst.
- (4) 1%ige Ammoniumchloridlösung.

##### a) Bestimmung des freien Eisenoxydes :

0,2~1 g gepulverter Probe wird in einem Becherglas mit 250 cc Lösung I übergossen. Das Becherglas wird auf dem siedenden Wasserbade bis auf 80~90° erhitzt. Darauf setzt man unter Umrühren 5 cc Ammonium-Ferrosulphatlösung hinzu und erhitzt noch ungefähr 10 Minuten. Nach Zusatz von 2,5 g Ammoniumchlorid lässt man auf Zimmertemperatur abkühlen, filtriert und wäscht mit der Ammoniumchloridlösung aus. Das Eisenoxyd ( $F_1$ ) im Filtrat wird bestimmt.

Das freie Eisenoxyd in der Probe ( $E$ ) =  $F_1 - A$

(A ist  $\text{Fe}_2\text{O}_3$  in 5 cc der Ammonium-Ferrosulphatlösung).

##### b) Bestimmung des nicht limonitischen und nicht hämatischen freien Eisenoxydes :

1 g Probe wird im Dunkeln mit 250 cc Lösung I bei Zimmertemperatur 1 Stunde lange ausgeschüttelt, nach Zusatz von 2,5 g Ammoniumchlorid über Nacht stehen gelassen, und das gelöste Eisenoxyd ( $F_{II}$ ) wird bestimmt.

##### c) Bestimmung des limonitischen Eisenoxydes :

0,5~1 g Probe wird in einem Becherglas mit 1 Lösung II übergossen, das Becherglas wird im Dunkeln bis auf 50° erwärmt, hierauf setzt man 5 cc Ammonium-Ferrosulphatlösung hinzu und erwärmt noch 20 Minuten bis auf 50°. Nach Zusatz von 10 g Ammoniumchlorid filtriert und bestimmt man das Eisenoxyd ( $F_{III}$ ) im Filtrat.

Limonitisches Eisenoxyd ( $E_l$ ) =  $F_{III} - F_{II} - A$

##### d) Ausrechnung des hämatischen Eisenoxydes :

Das hämatische Eisenoxyd ( $E_h$ ) =  $E - E_l - F_{II} = F_1 - F_{III}$

Die Bestimmung des freien limonitischen und hämatischen Eisenoxydes in

verschiedenen Bodenarten zeigt, daß sich das Eisen darin größtenteils (80~99% des in konz. Salzsäure löslichen Eisenoxydes) im freien Zustande befindet.

### **Effects of Certain Mineral Matters on the Growth of Root Nodule Bacteria. (Part III)**

(pp. 552~560)

By K. KONISHI, A. KAWAMURA and A. IMANISHI.

(Institute of Agr. Chem., Imp. University, Kyoto; Received May 7, 1940.)

Further experiments were conducted to ascertain the effects of chromium and manganese upon *Rh. meliloti*, by measuring  $Q_{O_2}$  and R. Q. in both nitrate mannitol solution and phosphate buffer. At the concentration of 0.001 or 0.01 per cent, sulphate of chromium exerted beneficial effects on the oxygen uptake by the organisms and also on their respiratory quotients, while sulphate of manganese did not.

Stimulating action of Cr-sulphate was remarkable as shown by early growth of the organisms on the mannitol media, where nitrogen was supplied with  $(NH_4)_2 \cdot SO_4$  as well as  $NaNO_3$  in different concentrations. Furthermore, the effect of Cr-sulphate was evident, when sucrose, succinic acid or acetic acid was used as the carbon source.

### **Über die Technische Citronensäuregärung. II. Mitteilung.**

(SS. 561~572)

Von M. NAKANO und K. KOBAYASI.

(The Institute of Research on Chemical Industry, Government-General of Taiwan, Japan;  
Received May 6, 1940.)

### **On the Absorption-Spectrum of Nucleotide.**

(pp. 573~574)

By Tetutaro TADOKORO and Naomoto TAKASUGI.

(Hokkaido Imperial University; Received May 6, 1940.)

## Report on the Shyotyu in Tyosen.

(pp. 575~580)

By Y. OHARA.

(Brewing Laboratory, Government General of Tyosen; Received May 16, 1940.)

The chemical constitution of 183 specimens of "Kurokozi-, Kyokusi- and Kasutori-Shyotyu" was investigated. Among these "Kurokozi-Syotyu" (Kaoliang, millet, rice, etc, are fermented for 1~2 weeks by "Kurokozi," *Asp. niger* and distilled) is now most usual in North Tyosen.

The following are the results of analysis.

Raw material	mg in 100 cc Syotyu					
	Alcohol (vol %)	Acid =acetic	Ester =ethyl	Furfural	Fusel oil	Aldehyde =aceto
" Kurozoki "	Kaoliang (52)	30.7	28	33	0.2	125
	Millet (17)	31.0	20	39	0.4	100
	Rice (11)	34.0	18	45	1.1	148
" Kyokusi " (10)		34.0	27	82	1.3	58
" Kasutori " (3)		33.6	31	137	1.5	—
						6.1

## Über die Jodometrie an Furfurol.

(SS. 581~585)

Von Matukitiro HAMADA und Kazuyuki MAEKAWA.

(Aus dem Agrikulturchemischen Institut der Kaiserlichen Kyushu- Universität  
in Fukuoka; Eingegangen am 18. 5. 1940.)

## Researches on Mechanical Wood Pulp.

(pp. 586~612)

### Part II. On a Laboratory Miniature Grinder.

By Mamoru WATANABE.

(Kyoto Imperial University; Received May 5, 1940.)

**Part III. On a Classifier for Wood Pulp.**

By M. WATANABE, Takesi YASUDA, Kazuaki KAWASE  
and Yositugu KIMURA.

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**Part IV. On Howan Howasun (*Larix dahurica* Turcz) of Manchoukuo  
as the Raw Material for Ground Wood Pulp.**

By M. WATANABE, T. YASUDA, K. KAWASE and Y. KIMURA.

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**Part V. On Yulin Sun (*Picea jezoensis* Carriere) of Manchoukuo  
as the Raw Material for Ground Wood Pulp.**

By M. WATANABE, T. YASUDA, K. KAWASE and Y. KIMURA.

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**Part VI. On Akamatsu (*Pinus densiflora* S. et Z.) of Nippon  
as the Raw Material for Ground Wood Pulp.**

By M. WATANABE, T. YASUDA, K. KAWASE and Y. KIMURA.

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**Chemical Studies on the Kikyo-root. (Report X)**

On the constitutional formulae of platycodigenin. (No. 3)

On the properties of a double bond and the  
oxygen atoms of platycodigenin.

(pp. 613~620)

By Magosaburo TSUJIMOTO.

(Agr. Chem. Laboratory, Kagoshima Imp. College of Agr. and Forestry ;

Received April 30, 1940.)

**SUMMARY.**

(1) Platycodigenin reduces an alkaline potassium permanganate solution, readily combines with bromine and iodine, gives a yellow colouration with tetrinitromethane. Catalytic reduction was unsuccessful. Therefore platycodigenin has a double bond, but it is very inactive.

(2) Platycodigenin possesses seven atoms of oxygen, two of them represented

by  $\text{-COOH}$ , and four of the others by  $(\text{OH})_4$ , and one atom of the last still unknown.

(3) From this view; Platycodigenin may be represented by the formulae  $\text{C}_{29}\text{H}_{46}\text{O}(\text{OH})_4 \cdot \text{COOH} \cdot \text{F}_1$ .